African Journal of Biotechnology Vol. 10(80), pp. 18458-18462, 14 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.896
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Competitive biosorption of different forms of lead [$Pb(NO_3)_2$ and $Pb(CH_3COO)_2$] on growth, biomass and proline in *Spirulina platensis* (Cyanophyta)

Selin Sayin[1]*, Ayşe Bahar Yilmaz[1], Nuray Ergün[2] and Funda Turan[1]

[1]Faculty of Fisheries, Mustafa Kemal University, 31200 Iskenderun, Hatay, Turkey.
[2]Department of Biology, Faculty of Science, Mustafa Kemal University, 31034 Antakya, Hatay, Turkey.

Accepted 17 October, 2011

Spirulina platensis **growth parameters [chlorophyll a (chl a) and dry-wet weight] effects on proline content, lead accumulation and the combined effect of the different forms of lead [Pb $(NO_3)_2$, Pb $(CH_3COO)_2$] and pH (6 to 8) were investigated for 192 h. The accumulation and form of lead were determined to be effective on growth parameters. While the highest chl *a* value (562.37 $\mu g l^{-1}$) and lead accumulation (58.74 $\mu g g^{-1}$) were found in medium with 30 $mg l^{-1}$ Pb $(CH_3COO)_2$ and pH 6, the highest proline content was found in 30 $mg l^{-1}$ Pb $(NO_3)_2$ and pH 6. This study depict an inverse relationship between lead accumulation in the test algae and low pH which suggests that proline might be produced at the expense of the material(s) required for the development of *S. platensis*.**

Key words: *Spirulina,* heavy metal, growth parameters, uptake.

INTRODUCTION

Metals are continuously released into the biosphere by volcanoes, natural weathering of rocks, and/or industrial activities such as mining, combustion of fossil fuels and the release of sewage (De-pledge et al., 1994). Toxic heavy metal contamination is a worldwide aquatic environmental problem and makes it necessary to develop efficient methods for proper removal of metal pollutants. The removal of toxic metal ions from waste-water using biological materials such as algae, bacteria, fungi and peat moss has proved to be commercially promising (Volesky, 1990). Especially, microalgae have been found to be very effective biosorbents in removing heavy metals from wastewater because of their large surface area and high binding affinity (Roy et al., 1993). Cell wall of microorganisms consisting mainly of poly-saccharides, proteins and lipids, offers many functional groups such as carboxylate, hydroxyl, thiol, sulphonate, phosphate, amino and imidazole groups binding metal ions (Ariff et al., 1999). Algae are able to tolerate certain concentrations of heavy metals through either the following general mechanisms: exclusion from cells and intracellular detoxification (Hall, 1981). The intracellular metal detoxification mechanisms comprise binding with metal-binding peptides and proteins, binding and precipitation within the cytoplasm and/or vacuole (Reed and Gadd, 1990) and sequestration with electron-dense polyphosphate granules. Many plants accumulate high concentrations of proline when treated with toxic concentrations of heavy metals (Costa and Morel, 1994). Proline plays important roles in osmoregulation, protec-tion of enzymes, stabilization of the machinery of protein synthesis, regulation of cytosolic acidity and scavenging of free radicals (Smirnoff and Cumbes, 1989). A few reports have also been made on algae (Fatma et al., 2007). Proline accumulation in plants also takes place in response to stresses such as salinity (Lutts et al., 1996), drought (Ibarra-Caballero et al., 1988), and low and high temperature (Naidu et al., 1991). Many researchers believe that proline accumulation is a symptom of injury which does not confer tolerance against metal or other stresses (Lutts et al., 1996).

A lot of studies in which *Spirulina* have been used as

*Corresponding author. E-mail: helenselin@yahoo.com or ssayin@mku.edu.tr. Fax: + 90 326 614 18 77. Tel: + 90 326 614 18 66.

Table 1. The composition of test mediums.

Experimental group's symbol	Medium		Initial pH
A	Zarrouk's medium	$Pb(NO_3)_2$	6
B	Zarrouk's medium	$Pb(NO_3)_2$	8
C	Zarrouk's medium	$Pb(CH_3COO)_2$	6
D	Zarrouk's medium	$Pb(CH_3COO)_2$	8
E	Zarrouk's medium	Control	6
F	Zarrouk's medium	Control	8
G	Zarrouk's medium	Control	9

biosorbents for heavy metals, may be useful for low cost industrial wastewater treatment where rapid removal of several heavy metals is needed (Rangasayatorn et al., 2002; Pane et al., 2008; Şeker et al., 2008). Some metals such as zinc, iron and manganese are involved in the metabolism of algae which are essential at low concentration rates for the activity of several enzymes; they accumulate in cells when they are present in excessive amount, causing toxicity and serious damage in metabolic pathways (Albergoni et al., 1980). Other metals such as lead and mercury, do not enter the metabolism of living beings, so they are considered non-essential and act as serious cell toxicants even at low concentration rates (Moreira, 2001).

Spirulina platensis (Cyanobacteria), a genus of blue-green photoautotrophic and unicellular microalgae, is an alternative source of protein, pigments, essential fat oil, vitamins, antioxidants and immunositimulants for both human food and animal (terrestrial, freshwater and marine) feed purposes (Belay et al., 1996). Also, it can be easily and cheaply recovered by filtration from the medium on account of its relatively large size. *S. platensis*, contain a variety of functional groups such as carboxyl, hydroxyl, sulphate and other charged groups which are responsible for metal binding (Li et al., 2006). It depends not only on the total and free metal concentrations, but also on the range of environmental and physiological factors influencing both chemical metal speciation and metal uptake by the cells (Pawlik, 2000). In this study, effects of different pH rates (6, 8 and 9) and the different forms of lead [$Pb(NO_3)_2$, and $Pb(CH_3COO)_2$] on lead accumulation on marine phytoplankton: *S. platensis* (as the most popular culture and toxicant bioabsorbant species) were examinated. In addition, the effects on the growth parameters (chlorophyll-a and dry-wet weight) and proline content were determined.

MATERIALS AND METHODS

S. platensis cultures

S. platensis-M2 obtained from the Plankton Culture Laboratory at University of Cukurova, Adana, Turkey, was used in this study. It was grown at 28 ± 2°C in Zarrouk's medium (Zarrouk, 1996) for eight days under white flourescent light (90 µmol photon m^{-2} s^{-1}) with 24 h illumination. All the chemicals were of analytical grade. All the procedures were performed under aseptic conditions using three triplicates.

Experimental plan

To examine the effect of heavy metal stress on the test organism (*S. platensis*-M2), inorganic form of Pb (as $Pb(NO_3)_2$) and organic form of Pb (as $Pb(CH_3COO)_2$) were added to the growth medium (fresh) (Zarrouk's medium) in calculated amount to obtain the final concentration of 30 mgl^{-1}. Each test was carried out in 2000 ml polyetilene bottles. The initial pH value of the solution was adjusted between 6, 8 and 9 by adding nitric acid (0.1M) or sodium hydroxide (0.1 M). The experiments were conducted at 28°C. Culture samples (10 ml) were collected by centrifugation (3000 rpm) at intervals of 1, 2, 3, 6, 12, 24, 96, 144 and 192 h. The composition of the test mediums, initial pH values and the chemical form of lead were used as shown in Table 1.

Sample analysis

Chlorophyll a values were determined according to the method of Parson and Strickland (1963). The amounts of cellular chl *a* were calculated using the highest cell abundance and chl *a* values (chlorophyll-a concentration; µ gl^{-1}) divided by cell abundance (cell L^{-1}) (Guillard, 1973).

Dry weight for samples containing 10 ml algal suspension was filtered through a filter of 47 mm (diameter) (Whatman GF/C) that was dried in microwave oven (105°C in 8 min) and weighed prior to filtration. The filter was put in a glass Petri dish in the oven under the earlier mentioned conditions. After cooling the filter in a desiactor (20 min), it was weighed again (Boussiba and Richmond, 1976). Proline content was determined according to method of Bates et al. (1973).

Determination of lead

The samples were filtered for metal analysis and digested using concentric nitric acid and hydrocloric acid according to the procedure described by Karadede and Ünlü (2000) using concentrated HNO_3/HCl (1:3 v/v). The digested samples were then diluted to 50 ml with 2.5% of HNO_3. The concentration of lead in the samples was determined by inductively coupled plasma atomic emission spectrometer (ICP-AES) (varian model – Liberty Series II). The absorption line used was 283.3 nm for lead, and metal concentrations were calculated in micrograms per gram wet weight (µg g^{-1} wet wt.).

Table 2. Experimental results of different lead mediums in 8 days incubation.

Parameter	A1	B2	C3	D4	E5	F6	G7
Biomass [wet weight (g/L)]	0.62±0.006^{a}	0.64±0.011^{ab}	0.63±0.020^{a}	0.61±0.011^{a}	0.67±0.010^{c}	0.68±0.010^{c}	0.66±0.020^{bc}
Biomass [dry weight (g/L)]	0.029±0.001^{a}	0.033±0.002^{a}	0.052±0.002^{b}	0.029±0.001^{a}	0.076±0.005^{d}	0.034±0.003^{a}	0.064±0.002^{c}
pH values	9.34±0.016^{a}	9.48±0.053^{bc}	9.45±0.010^{b}	9.51±0.007^{c}	9.95±0.037^{d}	9.48±0.009^{bc}	9.52±0.019^{c}
Pb uptake (µg/g)	31.30±0.002^{c}	33.46±0.577^{d}	58.74±0.5777^{e}	21.69±0.577^{b}	0.17±0.003^{a}	*N.D.	0.15±0.004^{a}
Proline (mg/g)	0.1477±0.0006^{g}	0.1007±0.0006^{f}	0.0337±0.002^{c}	0.0927±0.0006^{e}	0.0567±0.0006^{d}	0.0077±0.0006^{a}	0.0130±0.001^{b}
Chl *a* (µg/L)	477.17±3.945^{c}	413.61±3.004^{a}	562.37±3.216^{e}	449.85±6.992^{b}	740.63±1.464^{g}	523.79±5.633^{d}	668.53±18.312^{f}

Means within the same column with different letters are statistically significant (p□0.05); *N.D., not detected.

Statistical analysis

Data were analyzed statistically using one way analysis of variance (ANOVA). When significant treatment effects were detected, Duncan's multiple range test was used to identify specific differences among treatment means at a probability level of 5%.

RESULTS AND DISCUSSION

The results obtained for different pH and lead forms in cultures grown for 192 h (8 days) are shown in Table 2. As seen in Table 2, the results of this study proved that wet weight of the cells value of *S.platensis* was affected by the inorganic [Pb $(NO_3)_2$] and organic [Pb $(CH_3COO)_2$] Pb forms and when the results were compared, no difference was recorded between heavy metal groups ($p>0.05$). The highest dry weight values were recorded in E5 (0.076 ± 0.005 gL^{-1}) and C3 (0.052±0.002 gL^{-1}) groups. Moreover, the highest chl *a* values (740.63 ± 1.464 and 562.37±3.216 μgL^{-1}) were recorded in two groups (E5 and C3). Statistical differences between all studied groups were significant ($P<0.05$).

When data from the control groups were observed, it was seen that the highest growth was in E5 group at pH 6. In addition, wet weight results were consistent with the fact that initial pH value and Pb form affected algal growth. Similar to our results, a limitation of phytoplankton growth and photosynthesis at elevated pH levels was supported by Chen et al. (1994). The decrease in both growth rate and photosynthesis at high pH may have been caused by a number of different mechanisms including trace metal toxicity or limitation, reduced nutrient availability, or changes in the availability of carbon substrate (Chen et al., 1994).

The highest Pb uptake value was 58.74 µg/g at initial pH 6 (C3). The uptake was affected by pH value and Pb form. Many factors affect the behavior of metal sorption by algae, including pH, alkalinity, chelating (humic acid, EDTA and NTA), ion intensity, metal form and algal concentrations, temperature, light and exposure time. Perhaps, the most important single influencing parameter on metal uptake is pH (Gin et al., 2001).

Gin et al. (2001) reported in their study on the effect of the relationship between the initial pH and the final pH values on the metal uptake, that pH value increased following the uptake of the metal, while the increase in pH value led to metal precipitation, thus inhibiting the availability of the uptake and they also pointed out that initial low pH positively affected the metal uptake stage, which all support our study.

According to Xue et al. (1988), the absorption capacity of marine algae would be due to the functional groups present on the cell surface, mainly protein -COO-1 groups and functional chains of amino acids such as histidine, cysteine, aspartic acid and glutamic acid. In particular, carboxylic group was shown to bind lead (II) effectively, probably because of its bidentate structure. Bai and Abraham (2002) had revealed that acetylation of amino and hydroxyl groups of *Rhizopus nigricans* biomass obviously reduced the chromium biosorption. These facts indicate the important role played by carboxy, amino and hydroxyl groups in the biosorption of heavy metals.

The dry weight and chl *a* values in Al, B2 and D4 groups were lower than that of group C3, in consequence, organic form of Pb [$Pb(CH_3COO)_2$] might be less effective at pH 6. Algae may become tolerant to metal stresses through a variety of mechanisms, such as intracellular binding with metal ions (Rauser, 1984, Lee et al., 1996), extracellular exclusion of metal ions (Foster, 1977), or a reduction of metal uptake (Singh and Yadava, 1986). It was found that $Pb(NO_3)_2$ at pH 6 and 8 were more toxic than their CH_3COO_2 counterparts.

The fact that relatively high Pb accumulation in C3 group did not affect the growth of *S.platensis* indicates that there is no proportional relationship

between the higher metal accumulation and its toxicity. At the same, Pb was detoxified by a stress mechanism developed by the cells.

In this study, it was observed that heavy metals induced enhancement of intracellular proline content in the cyanobacterium, *S. platensis* (Table 2), making it more resistant to environmental stresses. The highest proline accumulation values (0.1477 ± 0.0006 and 0.1007 ± 0.0006 µg/g) were determined in A1 [$Pb(NO_3)_2$ at pH 6 and B2 [$Pb(NO_3)_2$) at pH 8 groups. There were significant differences in proline contents in the control and in other groups ($P<0.05$). While chlorophyll *a* content decreased with increasing proline content, it showed a rising trend under all heavy metals stresses tested (Table 2). It seems that an inverse correlation exists between proline accumulation and chlorophyll content under heavy metal stress.

The accumulation of proline was found to be directly related to the toxicity order of heavy metal forms [$Pb(NO_3)_2$ at pH 6 >$Pb(NO_3)_2$ at pH 8> $Pb(CH_3COO)_2$ at pH 8 > $Pb(CH_3COO)_2$ at pH6], that is, minimum accumulation was observed in cultures grown with $Pb(NO_3)_2$ at pH 8 and maximum accumulation in cultures grown with $Pb(NO_3)_2$ at pH 6.

Proline accumulation in higher plants under heavy metal stress has been reported by many workers (Alia and Saradhi, 1991; Bassi and Sharma, 1993a, b; Hanson and Burnet, 1994; Ashraf and Harris, 2004; Mansour et al., 2005). Although, also reported in algae (Wu et al., 1998), there are few reports (including those of the authors) on the effect of heavy metal stress on proline content in cyanobacteria (Wu et al., 1995; Choudhary et al., 2007). Fatma et al. (2007) reported increased proline in *Westiellopsis prolific*a (Cyanobacterium) under Pb and Cd heavy metal stress.

It was also found that environmental stress-induced proline accumulation is also correlated with metal forms and pH levels. From these findings, it can be concluded that proline appears to be a stress-induced substance in the cyanobacterium, *S. platensis*, and that it helps to resist adverse environmental conditions (forms of heavy metals and pH of culture medium). Thus, a cyanobacterium with the potential of synthesizing more proline, or a genetically engineered resistant cyanobacterium, may prove to be a promising candidate for the bioremediation of aquatic pollution.

REFERENCES

Albergoni V, Piccinni E, Coppellotti O (1980). Response to heavymetals in organisms-I. Excretion and accumulation of physiologicaland non physiological metals in *Euglena gracilis*. Comp. Biochem. Physiol. C 67: 121-127.

Alia P, Pardha SP (1991). Proline accumulation under heavy metal stress. J. Plant Physiol. 138: 554-558.

Ariff AB, Mel M, Hasan MA, Karim MIA (1999). The kinetics and mechanism of lead(II) biosorption by powderized *Rhizopus oligosporus*. World J. Microbiol. Biotech.15: 255-260.

Ashraf M, Harris PJC (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166: 3-16.

Bai RS, Abraham TE (2002). Studies on enhancement of Cr (VI) biosorption by chemically modified biomass of Rhizopus nigricans, Water Res. 36: 1224-1236.

Bassi R, Sharma SS (1993a). Changes in proline content accompanying the uptake of zinc and copper by Lemna minor. Ann. Bot. 72: 151-154.

Bassi R, Sharma SS (1993b). Proline accumulation in wheat seedlings exposed to zinc and copper. Phytochem. 33: 1339-1342.

Bates IS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Boyd CE (1979). Water Quality in Warmwater Fish Ponds. Auburn University Agricultural Experiment Station. p.482.

Belay A, Kato T, Ota Y (1996). Spirulina (Arthrospira): potential application as an animal feed supplement. J. Appl. Phycol. 8: 303-311.

Chen CY. EG., Durbin, (1994). Effects of pH on the growth and carbon uptake of marine phytoplankton. Mar. Ecol. Progr. Ser. 109: 83-94.

Chonjnacka K, Chonjnacki A, Górecka H (2005). Biosorption of Cr, Cd and Cu ions by blue-green algae Spirulina sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere, 59: 75-84.

Costa G, Morel JL (1994). Water relations, gas exchange and amino acid content in cadmium-treated lettuce. Plant Physiol. Biochem. 32: 561-570.

Choudhary M, Jetly UK, Khan A, Zutshi S, Fatma T (2007). Effect of heavy metal stress on proline and malondialdehyde and superoxide dismutase activity in the cyanobacterium *Spirulina platensis* S-5. Ecotoxicol. Environ. Saf. 66: 204-209.

De-Filippis LF, Hampp R, Ziegler H (1981). The effects of sublethal concentrations of zinc, cadmium and mercury on Euglena growth and pigments. Z. Pflanzen. Physiol. 101: 37-47.

Depledge MH, Weeks JM, Bjerregaard P (1994). Metals. In: Pete Callow (ed) Handbook of ecotoxicology, 2. Blackwell Scientific Publications, pp. 79-105.

Fatma T, Khan MA, Choudhary M (2007). Impact of environmental pollution on cyanobacterial proline content. J. Appl. Phycol. 19: 625-629.

Foster PL (1977). Copper exclusion as a mechanism of heavy metal tolerance in a green alga. Nature (Lond.) 269: 322-323.

Gin KYH, Tang YZ, Aziz ZA (2001). Heavy metal uptake by Algae.Photosynthetic Microorganisms in Environmental Biotechnology. Springer, Hong Kong, pp. 137-168.

Gong R, Ding Y, Liu H, Chen Q, Liu Z (2005). Lead biosorption and desorption by intact and pretreated Spirulina maxima biomass. Chemosphere, 58: 125-130.

Guillard RRL (1973). Division Rates in Handbook of Phycological methods (J. R. ETEIN editör). Culture Methods and Growth Measurements, Chambridge Universty Press, Chambridge. pp. 289-311.

Hall A (1981). Copper accumulation in copper-tolerant and nontolerant populations of the marine fouling alga, *Ectocarpus siliculosus* (Dillw.) Lyngbye. Botanica Marina. 29: 223-228.

Hanson AD, Burnet M (1994). Evolution and metabolic engineering of osmoprotectant accumulation in higher plants. In: Cherry JH (ed) Biochemical and cellular mechanisms of stress tolerance in plants. Springer, Berlin, pp. 291-302.

Ibarra-Caballero J, Villanueva-Verduzco J, Molina-Galam J, Jimenez SD (1988). Proline accumulation as a symptom of drought stress in maize: a tissue differentiation requirement. J. Exp. Bot. 39: 889-897.

Karadede H, Unlu E (2000). Concentrations of some heavy metals in water, sediment and fish species from the Ataturk Dam Lake (Euphrates), Turkey. Chemos. 41: 1371-1376.

Lee JG, Ahner BA, Morel FMM (1996). Export of cadmium and phytochelatin by the marine diatom Thalassiosira weissflogii. Environ. Sci. Technol. 30: 1814-1821.

Li ZY, Guo SY, Li L (2006). Study on the process, thermodynamical isotherm and mechanism of Cr(III) uptake by *Spirulina platensis*, J. Food Eng. 75: 129-136.

Lutts S, Kinet JM, Bouharmont J (1996). Effects of various salts and of mannitol on ion and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) callus cultures. J. Plant Phys. 149:186-195.

Mansour MMF, Salama KHA, Ali FZM, Abou HAF (2005). Cell and plant responses to NaCl in Zea mays L. Cultivars differing in salt tolerance. Gen Appl. Plant. Physiol. 31: 29-41.
Moreira JC (2001). Threats by heavy metals: human and environmental contamination in Brazil. Sci. Total Environ. 188: 61-71.
Naidu BP, Paleg LG, Aspinall D, Jenning AC, Jones GP (1991). Amino acid and glycine betaine accumulation in cold stressed wheat seedlings. Phytochemistry, 30: 407-409.
Parson TR, Strickland JDH (1963). 2 Discussion of spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. J. Mar. Res. 21: 115-163.
Pawlik-Skowro SB (2000). Relationships between acid-soluble thiol peptides and accumulated Pb in the green alga *Stichococcus bacillaris*. Aquat. Toxicol. 50: 221-230.
Rangsayatorn N, Upatham ES, Kruatrachue M, Pokethitiyook P, Lanza GR (2002). Phytoremediation potential of Spirulina (Arthrospira) platensis: biosorption and toxicity studies of cadmium, Environ. Pollut. 119: 45-53.
Rauser WE (1984). Copper-binding protein and copper tolerance in Agrotis gigantea. Plant Sci. Lett. 33: 239-247.
Reed RH, Gadd GM (1990). Metal tolerance in eukaryotic and prokaryotic algae. In: Shaw AJ, ed. Heavy metal tolerance in plants: evolutionary aspects. Boca Raton, FL, USA: CRC Press. pp. 105-118.
Roy D, Greenlaw PN, Shane BS (1993). Adsorption of heavy metals by green algae and ground rice hulls. J. Environ. Sci. Health A. 28: 37-50.
Singh SP, Yadava V (1986). Cadmium tolerance in the cyanobacterium Anacystis nidulans. Biol. Zentralbl. 105: 539-542.
Smirnoff N, Cumbes QJ (1989). Hydroxyl radical scavenging activity of compatible solute. Phytochemistry, 28: 1057-1060.
Volesky B (1990). Removal and recovery of heavy metals by biosorption. In: Volesky B (Ed.), Biosorption of Heavy Metals. CRC Press, Boca Raton, pp. 173-198.
Wu JT, Chang SC, Chen KS (1995). Enhancement of intracellular proline level in cells of Anacystis nidulans (cyanobacteria) exposed to deleterious concentrations of copper. J Phycol. 31: 376-379.
Wu JT, Hsieh MT, Kow LC (1998). Role of proline accumulation in response to toxic copper in Chlorella sp.(Chlorophyceae) cells. J. Phycol. 34: 13-117.
Xue H, Stumm W, Sigg L (1988). The binding of heavy metals to algal surfaces.Water Res. 22: 917-926.
Zarrouk C (1996). Contribution a l'etude d'une cyanophycee de divers facteurs physique et chimiques sur la croissane et photosynthese de *Spirulina maxima*. Geitler, Ph.D. thesis, in French, University of Paris, France.